American Journal of Hematology 59:79–82 (1998)

Analysis of Clonality Using X-Linked Polymorphisms in a Patient With Multiple Myeloma and Myelofibrosis

James P. Stevenson,[1]* Roland Schwarting,[2] and Stephen J. Schuster[1]

[1]Cardeza Foundation for Hematologic Research, Thomas Jefferson University, Philadelphia, Pennsylvania
[2]Department of Pathology and Cell Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania

We describe a patient who presented with a neutrophilic leukocytosis, normal karyotype, and IgAλ multiple myeloma. One year after diagnosis she developed diffuse myelofibrosis as well as multiple lytic lesions of bone. Given the myeloproliferative features of her case, the clonality of her peripheral leukocytes was determined prior to treatment. Analysis of X-chromosome inactivation at the X-linked human androgen-receptor gene locus (HUMARA) proved that granulopoiesis was polyclonal. Subsequent treatment of the myeloma reversed with myelofibrosis and normalized her WBC count. This is the first case of multiple myeloma with myelofibrosis in which a concomitant clonal myeloproliferative disease was ruled out at a genetic level. The myeloproliferative features in this case are presumed to be induced by cytokines produced by the plasma cell clone. Am. J. Hematol. 59:79–82, 1998. © 1998 Wiley-Liss, Inc.

Key words: multiple myeloma; myelofibrosis; clonality; X-chromosome inactivation

INTRODUCTION

An association between multiple myeloma (MM) and the myeloproliferative syndromes has long been recognized, dating back to the early 1900s. Concomitant cases of MM with polycythemia vera (PV), chronic granulocytic leukemia (CGL), and idiopathic myelofibrosis or agnogenic myeloid metaplasia (IF) have been repeatedly described, and often were felt to represent the simultaneous occurrence of distinct clinical entities. This distinction was previously based on clinical, morphologic, and laboratory features.

The advent of molecular biology has confirmed that myeloproliferative disease and multiple myeloma are clonal proliferations of cells arising from different hematopoietic elements, i.e., the pluripotent stem cell in the myeloproliferative syndromes, and the lymphoid/plasma cell compartment in MM. The development of clonal analysis using X-linked DNA polymorphisms in informative females [1] provides a means to define the origin of these processes at a molecular level and address the question posed in cases of simultaneous MM and IF: one disorder or distinct entities?

We present a case of MM with IF analyzed using X-linked polymorphisms for clonal derivation of myeloid lineage cells: this represents the second reported case of myeloma with myeloproliferative features to be subjected to this type of analysis.

CASE REPORT

A 60-year-old woman without significant medical history presented in December 1995 with persistent neutrophilic leukocytosis without an obvious infectious source. In August 1995, the WBC was 21.0×10^9/L with differential white cell count: neutrophils 80%, band forms 5%, lymphocytes 11%, monocytes 4%. Intermittent night sweats developed during the month prior to our evaluation but the patient was otherwise completely asymptomatic. No hepatosplenomegaly or lymphadenopathy was noted on examination.

In December 1995, the WBC was 19.3×10^9/L with neutrophils 62%, bands 15%, metamyelocytes 1%, myelocytes 3%, lymphocytes 14%, monocytes 4%, basophils 1%; hemoglobin was 12.6 g/dL and platelet count 309×10^9/L. Serum alkaline phosphatase was elevated to

*Correspondence to: Dr. James P. Stevenson, University of Pennsylvania, Presbyterian Medical Center, Medical Arts Building-Hematology/Oncology, 51 North 39th Street, Philadelphia, PA 19104.

Received for publication 8 December 1997; Accepted 8 April 1998

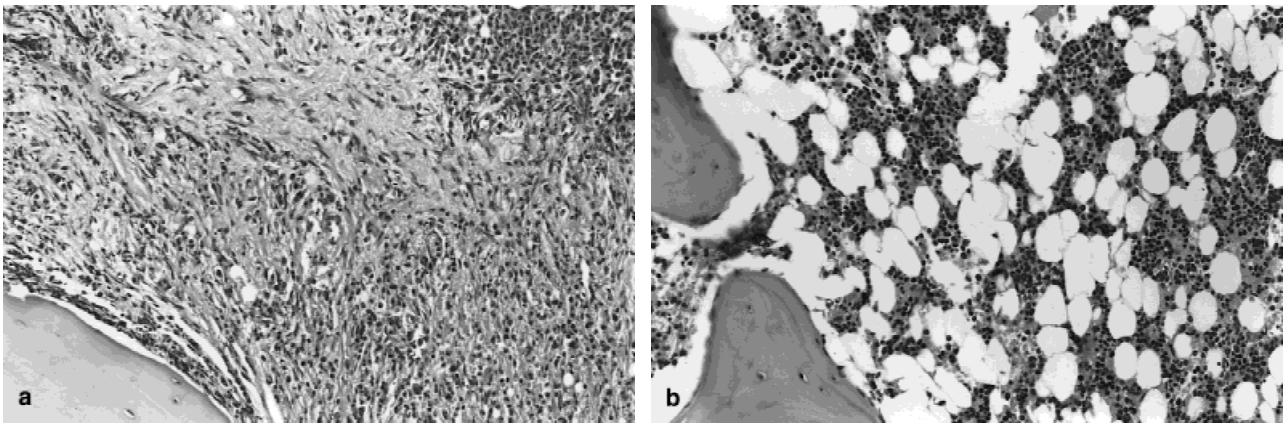

Fig. 1. Photomicrographs of hematoxylin and eosin stained bone marrow biopsy sections from the patient before (a) and after (b) systemic chemotherapy (×40). Extensive marrow fibrosis noted prior to therapy disappeared following three cycles of VAD chemotherapy and normal hematopoiesis returned upon recovery.

174 IU/L (normal range 35–115 IU/L), sedimentation rate 33 mm/hr, and leukocyte alkaline phosphatase score increased to 241 (normal range 20–120). Serum calcium and creatinine were normal. A monoclonal gammopathy was noted on serum protein electrophoresis (SPEP), identified as IgAλ by immunofixation and measured 2.1 g/dL by nephelometry. There was no Bence-Jones proteinuria. Serum β_2-microglobulin was normal. Serum IgG and IgM levels were decreased to 458 and 49 mg/dL, respectively (normal ranges 723–1,683 and 63–277 mg/dL).

Bone marrow aspirate showed myeloid hyperplasia as well as 6% plasma cells. Trephine biopsy was 90% cellular with increased myelopoiesis and marked granulopoiesis. Scattered syncytia of plasma cells expressing IgAλ by immunohistochemical staining were noted. Karyotype was normal 46XX. No lytic or sclerotic lesions were seen on skeletal survey, and liver and spleen size were normal by ultrasound measurement.

Based upon these findings, the diagnosis was IgAλ MM, stage I, with a reactive leukocytosis. Physical examination, serum chemistries, and SPEP were followed monthly and remained stable. In July 1996, the patient developed severe lumbar pain. Paraprotein level was 2.4 g/dL, hemoglobin 12.0 g/dL, platelet count 295×10^9/L, and WBC 22.2×10^9/L. Repeat skeletal survey was normal. MRI of the lumbosacral spine showed multiple levels of disc herniation without bony lesions or abnormal marrow signal. The patient's symptoms were temporarily relieved by an epidural cortisone injection, but soon returned. Bone marrow examination was repeated in November 1996 and was again hypercellular (8% plasma cells); however, immature plasmablasts as well as dense myelofibrosis with nests of plasma cells were now noted. Peripheral blood red cell morphology was normal; no fragmented cells or nucleated precursors were observed. Repeat abdominal ultrasound revealed mild hepatomegaly and splenomegaly. New lytic lesions in the skull and L2 vertebral body were evident on X ray. Diffuse marrow signal abnormality was now present on repeat lumbosacral MRI.

Clonality of the patient's peripheral blood leukocytes was assessed initially at the X-linked G6PD/p55 genes; however, she was not informative at these loci [2]. Analysis at the X-linked human androgen receptor locus (HUMARA) was performed as previously described [3]. The patient's DNA was informative at this locus and her peripheral leukocytes were found to be of polyclonal origin, with a ratio of paternal/maternal alleles of 1.4 in polymorphonuclear leukocytes.

Systemic chemotherapy consisting of vincristine and doxorubicin by continuous infusion and high-dose dexamethasone (VAD) was administered for three monthly courses. The patient's symptoms resolved completely and her WBC count fell to 4.6×10^9/L. Monoclonal gammopathy was no longer detectable on SPEP, although a faint IgAλ monoclonal band was detected by immunofixation. Repeat bone marrow examination was normocellular with resolution of the previously noted myelofibrosis (Fig. 1). Immunohistochemical stains revealed no evidence of residual myeloma with only scattered kappa and lambda staining noted. High-dose melphalan was administered with autologous stem cell rescue in May 1997, to which the patient had a complete response. Bone marrow morphology and cellularity 6 months post-stem cell rescue were normal and there is presently no evidence of the previously noted IgAλ monoclonal band by immunofixation.

DISCUSSION

Cases of MM with myeloproliferative features have been described throughout this century. Reports of the

synchronous occurrence of MM with PV, CGL, or IF caused some to suspect concomitant but separate clonal disorders, while others postulated the presence of one clonal disorder with distinct secondary phenomena.

Two such cases were described in 1964 [4]. One patient had features of MM and PV, another MM and IF. The authors suggested a working ''interdependence'' between these entities and postulated a common pluripotent cell origin. Another two cases of MM and IF were subsequently reported and presumed to be coincidental clonal disorders [5]. In yet another series, five cases of MM associated with myelofibrosis but without myeloid metaplasia were described; the authors concluded that the fibrosis was secondary to the MM in these cases [6]. They suspected the myelofibrosis was distinct from previously described cases of MM associated with both myelofibrosis and myeloid metaplasia. Other authors [7,8] felt that the presence or absence of myeloid metaplasia in cases of MM with myelofibrosis did not differentiate between related and coincident phenomena. They proposed that the reversibility of IF upon treatment of MM observed in their series served as proof that this process was secondary to the MM and likely cytokine-induced. One report [9] of a case of IgAλ MM associated with IF that was not reversible with alkylator-based therapy adds some confusion to the picture, but the authors still believed the IF to be cytokine-induced in the setting of a clonal plasma cell dyscrasia.

There are multiple reports in the literature of MM associated with chronic neutrophilic leukemia (CNL). The coexistence of this exceedingly rare myeloproliferative disorder with MM should be almost unheard of. Two such cases have been described [10] and the occurrence of two disorders arising from a single transformed pluripotent stem cell was proposed. However, markedly elevated serum G-CSF and IL-6 levels were documented in another case of IgGκ MM associated with CNL [11], supporting the authors' hypothesis that the leukocytosis was secondary in nature. This controversy was finally laid to rest by the description of a case of IgGλ myeloma and associated CNL in which clonal analysis using the X-linked probe M27β revealed the patient's neutrophils to be polyclonal [12].

Bone marrow myelofibrosis is commonly described in a broad range of both malignant and non-malignant conditions. Its occurrence in and relation to the clonal myeloproliferative diseases has been suggested to represent a process secondary to the malignant disease and not itself of clonal origin. Indeed, this has been proven at the molecular level, as marrow fibroblasts in myeloproliferative disease are not clonal by X-linked analysis [1]. Presumed cytokines involved in the development of marrow fibrosis include PDGF and TGF-β. Malignant plasma cells express TGF-β, as well as G-CSF, in vivo [13,14].

The patient described here represents the first case in the literature of MM with myelofibrosis studied in terms of clonality. The polyclonality of the patient's leukocytes based on X-linked polymorphism analysis at the HUMARA locus support the hypothesis that myelofibrosis as well as leukocytosis in this case was secondary to the clonal proliferation of malignant plasma cells. The fact that the patient did not have classic features of myeloid metaplasia, such as leukoerythroblastosis and massive hepatosplenomegaly, does not necessarily suggest that previously described cases with these features still represent concurrent clonal disorders. Repeat abdominal ultrasound exam in this patient documented mild hepatosplenomegaly synchronous with the discovery of marrow fibrosis. One may speculate that worsening organomegaly as well as leukoerythroblastosis and extramedullary hematopoiesis may have developed as a consequence of progressive fibrosis had this woman not received chemotherapy. Thus, the clinical presentation of this patient allowed the observation of a single malignancy in evolution and its subsequent definition at a clonal level.

ACKNOWLEDGMENTS

We are grateful to Dr. D. Gary Gilliland for performing the HUMARA assay and for critical review of this manuscript.

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